REMARKS

Reconsideration and withdrawal of the rejections of the claims, in view of the remarks presented herein, is respectfully requested. Claims 1-29 were previously cancelled. The pending claims are claims 30-55.

The 35 U.S.C. § 103 Rejection of the Claims

The Examiner rejected claims 30-32, 34-41, 43-50 and 52-55 under 35 U.S.C. § 103(a) as being unpatentable over McLaughlin *et al.* (*Journal of Endotoxin Research*, *1*, 165 (1994)) in view of Alexander *et al.* (*J. of Bacteriology*, *176*, 7079 (1994)). In addition, the Examiner rejected claims 30, 33, 39, 42, 50 and 51under 35 U.S.C. § 103(a) as being unpatentable over McLaughlin *et al.* and Alexander *et al.* in view of Świerzko *et al.* (*Infection and Immunity*, *61*, 3216-3221 (1993)).

1. Pending Claims

Claim 30 is directed to a process for the production of a *H. influenzae*-specific LOS that involves growing gram-negative bacteria containing (i) a core lipid structure containing a terminal heptose, (ii) a DNA sequence having a *rfe* gene, and (iii) an isolated DNA sequence with a lipooligosaccharide-synthesis gene G (*lsgG*) from *H. influenzae*. The *lsgG* encodes LsgG, which regulates *rfe*, such that *H. influenzae*-specific LOS is synthesized by the addition of an acceptor molecule to the terminal heptose molecule, and recovering the *H. influenzae*-specific LOS from the culture medium.

Claim 39 is directed to a process for the production of a complex carbohydrate involving growing in a culture medium gram-negative bacteria with a core lipid structure containing a terminal heptose and (i) a DNA sequence having a *rfe* gene and (ii) an isolated DNA sequence having a *lsgG* from *H. influenzae*, wherein *lsgG* encodes LsgG, and wherein *rfe* is regulated by LsgG such that a complex carbohydrate is synthesized by the addition of an acceptor molecule to the heptose molecule, and recovering the complex carbohydrate from the culture medium.

Claim 48 is directed to a method of modifying a terminal heptose of a lipopolysaccharide (LPS) or lipooligosaccharide (LOS) core structure of a gram-negative bacterial species

containing an *rfe* gene. The method involves regulating the *rfe* gene with a protein encoded by an isolated *lsgG* gene from *H. influenzae* such that N-acetyl glucosamine is added onto the terminal heptose.

Applicants' claims are directed to the enzymatic synthesis of complex carbohydrates. These claimed processes involve the use of an *rfe* (which encodes UDP-GlcNac:Undecaprenol GlcNac-1 phosphate transferase), and *lsgG* (which encodes an LsgG regulatory protein). Applicants disclose that UDP-GlcNac:Undecaprenol GlcNac-1 phosphate transferase catalyzes the transfer of N-acetyl glucosamine onto the carrier lipid undecaprenol phosphate. Applicants further disclose that expression of *rfe* is controlled by LsgG. By exploiting this interaction between *rfe* and *lsgG*, Applicants disclose that complex carbohydrates, such as a *H. influenzae*-specific LOS, can be synthesized onto a core lipid structure containing a terminal heptose.

2. McLaughlin *et al.* in view of Alexander *et al.*

McLaughlin *et al.* discussed the sequence of the *lsg* locus from *H. influenzae*. They identified eight open reading frames (ORFs) and performed transposon mutagenesis to try to begin to determine what, if any, function the products deduced from the ORF sequences might have. The authors of the paper did not have success in identifying the function of the deduced protein sequences encoded by the ORFs.

The Examiner asserts that McLaughlin *et al.* teach that "various sugar transferases expressed from the lsg are responsible for the modification of the existing *E. coli* LPS (page 172)" (page 3 of the Office Action). Applicants would like to draw the Examiner's attention to the full passage at page 172 of McLaughlin *et al.*, which states that

> "it is most probably various sugar transferases expressed from the Hib *lsg* locus that are responsible for these modification of the existing *E. coli* LPS. . . . Thus, it is likely that the *lsg* locus should contain a series of genes coding for sugar transferases. The sequence analysis of the 7.4 kb fragment and database search for the proteins homologous with the 8 ORFs, however, failed to show significantly high homology to any known sugar transferase, and it was not possible to deduce the functions of the products of the ORFs based on the sequence homology." (emphasis added)

McLaughlin *et al.* later state that "future studies will be directed at defining the functions of the proteins expressed by the ORFs within this locus" (page 174). Thus, when the statement quoted by the Examiner is read in context of the full passage from McLaughlin *et al.*, it becomes clear that McLaughlin *et al.* expected one or more of the ORFs to encode a sugar transferase, but that they could not find any significantly high homology to any known sugar transferase. Thus, at the time of pubication, they could not determine the functions of the putative products encoded by the ORFs.

The Examiner states that LsgG is among the eight ORFs taught by McLaughlin *et al.*, and alleges that since the LsgG protein of McLaughlin *et al.* and the LsgG protein of the instant invention is identical, regulation of *rfe* by LsgG is an inherent property of LsgG. A retrospective view of inherency is not a substitute for some teaching or suggestion which supports the selection and use of the various elements in the particular claimed combination. *In re Newell*, 891 F.2d 899, 13 U.S.P.Q.2d 1248, 1250 (Fed. Cir. 1989). In deciding that a novel combination would have been obvious, there must be supporting teaching in the prior art. *Id.* Applicants respectfully assert that it was not until the experiments were performed by the present inventors that it was discovered that the LsgG protein was encoded by one of the eight ORFs. As discussed above, McLaughlin *et al.* could not determine the functions of the putative products encoded by the ORFs. Thus, McLaughlin *et al.* could not teach which specific ORF encoded LsgG. This information would be needed before the ORF could be isolated and then used to produce a *Haemophilus influenzae*-specific LOS (claim 30) or a complex carbohydrate (claim 39), or to modify a terminal heptose of a lipopolysaccharide (LPS) or lipooligosaccharide (LOS) core structure (claim 48).

Further, as conceded by the Examiner at page 3 of the October 6, 2003 Office Action, McLaughlin *et al.* does not teach a process of making lipooligosaccharide (LOS) using an rfe enzyme.

Alexander *et al.* do not remedy the deficiencies of McLaughlin *et al.* At pages 3 and 5 of the October 6, 2003 Office Action, the Examiner alleges that Alexander *et al.* teach that *rfe* is "essential for the first step in the biosynthesis of lipooligosaccharide" and cites to page 7079 of

Alexander *et al.* to support this proposition (emphasis added). Applicants respectfully submit that Alexander *et al.* disclose that *rfe* is involved in the biosynthesis of enterobacterial common antigen (ECA), the biosynthesis of the O7 repeat of *E. coli* as well as other O-specific polysaccharides, *e.g.*, O18, O75 and O111 (page 7079, right hand column).

It is well-known in the art that lipooligosaccharide (LOS) molecules are not the same as ECA, O7 repeats or other O-specific polysaccharides. *See, e.g.*, Preston *et al.*, Critical Reviews in Microbiology, 22:139-180 (1996) (copy enclosed). The following excerpt from Preston *et al.* (pages 139-141, 145 (emphasis added)) gives an overview of the distinctions between LOS and these other types of molecules:

> Enteric Gram-negative bacteria living in the hydrophobic environs of the gastrointestinal tract have relatively hydrophilic surfaces due to the repetitive O-side chains of the enteric LPS. . . . In contrast, bacteria that reside on the respiratory and genital mucosal surfaces have outer membrane surfaces that are relatively hydrophobic and are susceptible to solubilization by bile. These bacteria have evolved a unique set of surface glycolipids that lack O-antigens and have been termed Lipooligosaccharides (LOSs). Although LOS is expressed predominantly on mucosal bacteria, including members of the genera *Neisseria, Haemophilus, Bordetella,* and *Branhamella*, it can also be expressed on enteric bacteria, for example *Campylobacter jejuni* and *Campylobacter coli* strains. LOS are analogous to the LPS found in other Gram-negative families. They share similar lipid A structures with an identical array of functional activities, but they lack O-antigen units with the LOS oligosaccharide structures limited to around ten saccharide units (Figure 1). Other differences between LPS and LOS are found in the genetics of their biosyntheses in that genes responsible for assembly of the core regions of LOS do not appear to be clustered in operons as they are in enteric bacteria. . . . The use of the term LOS to describe these glycolipids has generated some controversy and it is not universally used. As we learn more about these glycolipids, the more evidence accumulates that they are not rough versions of enterobacterial LPS (r-type) as previously reported. LOS are distinct structures with functions and features that have been appropriately adapted to allow the pathogenic bacteria that express them to be successful human pathogens.

Thus, it is well-known in the art that LOS molecules are not O-specific polysaccharides. Exhibit 1A (attached) depicts the chemical structure of an Lipopolysaccharide (LPS), and Exhibit 1B depicts the chemical structure of a Lipooligosaccharide (LOS). As can be seen in the

diagram, O-antigen is a subunit of LPS, but is not present in LOS. *See also*, Figures 1A and 1B in Preston *et al.*

Thus, it is respectfully submitted that a *prima facie* case of obviousness has not been established. Neither McLaughlin *et al.* nor Alexander *et al.*, either alone or in combination, disclose or suggest all the claim limitations. There is no teaching in McLaughlin *et al.* regarding the function of any products encoded by the *lsg* gene cluster, nor does it provide any suggestion or motivation to the art worker to modify the reference so as to arrive at the claimed invention. Additionally, Alexander *et al.* provides no motivation to one of ordinary skill in the art to modify McLaughlin *et al.*, because Alexander *et al.* relates to the role *rfe* plays in the formation of O-specific polysaccharide and ECA. There is nothing in either of McLaughlin *et al.* or Alexander *et al.* that teaches or suggests that LsgG regulates rfe such that a *H. influenzae*-specific LOS or complex carbohydrate may be synthesized, or to modify the terminal heptose of a LPS or LOS.

Moreover, neither McLaughlin *et al.* nor Alexander *et al.* provide to the art worker a reasonable expectation of success that *rfe* and *lsgG* could be employed to produce an *H. influenzae*-specific LOS, produce a complex carbohydrate, or to modify a terminal heptose of a core LPS or LOS structure. Strains derived from an *E. coli* K12 background (*e.g.*, JM109, DH5α) do not produce O-antigen extensions from the core regions of their lipopolysaccharide. This is considered to be due to the absence of genes in the *rfb* gene cluster (*see*, for example, Stevenson *et al.*, J. Bacteriology, 176, 4144-4156 (1994) (a copy is enclosed herewith). Therefore, any substitution of its core region by sugars from such a diverse species as *H. influenzae* would be unexpected. Moreover, the art worker would have no reasonable expectation of success that a *H. influenzae* gene cluster could assemble carbohydrates on an *E. coli* core region in the appropriate configuration to replicate different *Haemophilus* epitopes (*e.g.*, epitopes designated by monoclonal antibodies such as 6E4 and 3F11 disclosed in Examples 2-3 and 6 of Applicants' specification).

In addition, it is respectfully submitted that the art worker would not reasonably expect that *rfe* could be used to produce *H. influenzae*-specific LOS by the presently claimed method. As discussed above, *rfe* is involved in the assembly of the O-antigen repeat units, which are then

assembled into the O-antigen (Alexander *et al.* at page 7079). These polymeric repeats are then transported to the *E. coli* surface and ligated by a separate enzyme to the core region of the LPS in the periplasmic space of *E. coli* (Raetz et al., Annu. Rev. Biochem., 71, 635-700 (2002)). This ligation is usually to a hexose or an N-acetylhexosamine residue on the core region (Raetz et al., Annu. Rev. Biochem., 71, 635-700 (2002)). Applicants submit that an art worker would have no reasonable expectation that (1) a *H. influenzae*-specific LOS can be assembled on a N-acetylglucosamine-undecaprenol structure synthesized by *E. coli rfe*; and that (2) the *H. influenzae* carbohydrate structure synthesized through *rfe* ion the undecaprenol pyrophosphate lipid carrier is ligated onto the terminal heptose of the core region. It should also be noted that others have cloned bacterial LPS biosynthesis genes into *E. coli* strains of the same pedigree disclosed in Applicants' specification and have never described assembly of that strain's LPS structure in *E. coli* K12 assisted by *rfe* (Raetz et al., Annu. Rev. Biochem., 71, 635-700 (2002)).

It is respectfully submitted that the presently claimed invention is not obvious in view of the cited art. Therefore, Applicants respectfully request withdrawal of the 35 U.S.C. § 103(a) rejection of the pending claims over McLaughlin *et al.* in view of Alexander *et al.*

3. McLaughlin *et al.* and Alexander *et al.* in view of Świerzko *et al.*

McLaughlin *et al.* and Alexander *et al.* are discussed above.

Świerzko *et al.* do not remedy the deficiencies of McLaughlin *et al.* or Alexander *et al.* Świerzko *et al.* disclose the serological characterization of antisera collected from rabbits immunized with heat-killed *Salmonella minnesota* R4 chemotype Rd_2P^- (page 3218). In a passive hemolysis assay, Świerzko *et al.* disclose that the smallest structure recognized by the antisera was Hep-Kdo-$GlcNhm_2$, and the smallest structure reacting in an EIA was Hep-Kdo disaccharide. However, there is nothing in Świerzko *et al.* that teaches or suggest a process for the production of an *H. influenzae*-specific LOS, as recited in claim 30. Moreover, Świerzko *et al.* do not teach or suggest producing and recovering a complex carbohydrate using *rfe* and *lsg*, as recited in claim 39. Nor does Świerzko *et al.* teach or suggest modifying a terminal heptose of an LPS or LOS core structure using *rfe* and *lsg*, as recited in claim 48. Therefore, the pending

claims are not obvious over the combination of McLaughlin *et al.*, Alexander *et al.* and Świerzko *et al.*

Withdrawal of the 35 U.S C. § 103(a) rejection is respectfully requested.

Conclusion

Applicants respectfully submit that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicants' attorney (612-337-2540) to facilitate prosecution of this application.

Enclosed is a check in the amount of $1,720.00 to cover the RCE fee ($770) and the Petition for Extension of Time fee ($950). Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

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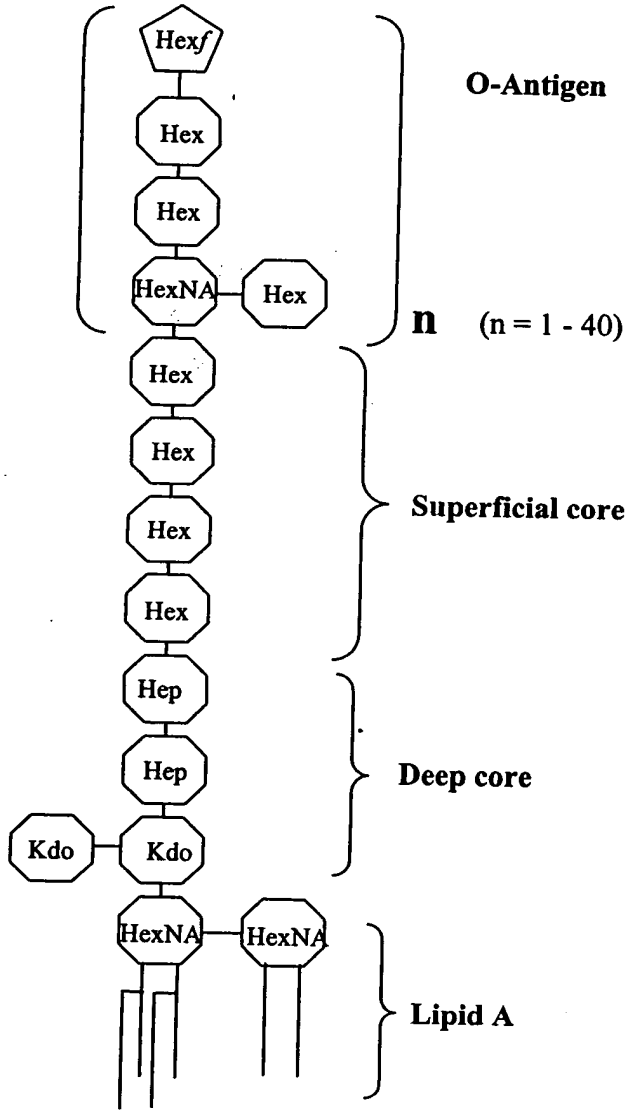
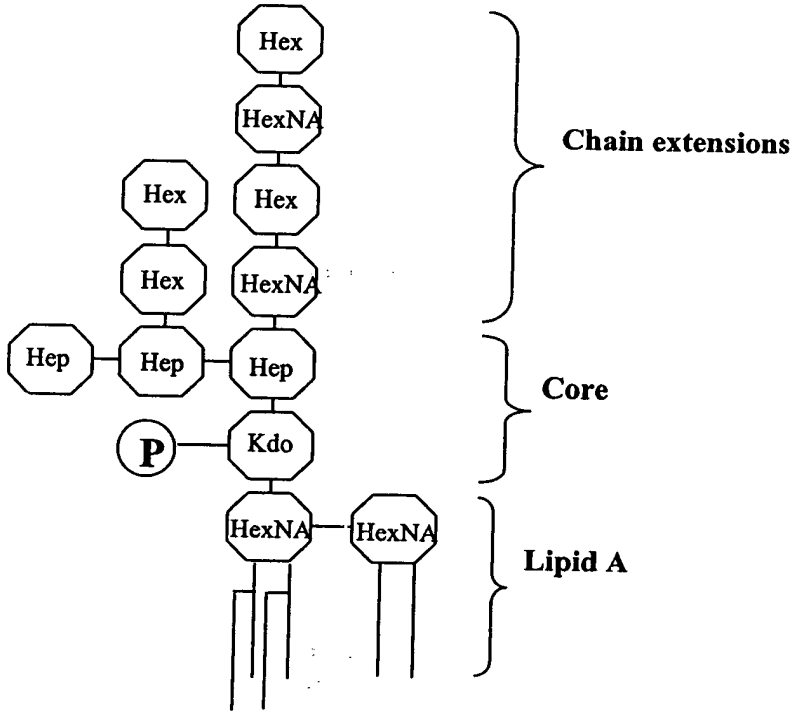
Exhibit 1

A. Enterobacterial Lipopolysaccharide



Size - up to 65,000 Daltons

B. *Haemophilus* Lipooligosaccharide



Size - > 6,000 Daltons